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Stratification Requirements for Germination of Western Larch (*Larix occidentalis* Nutt.) Seed

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Abstract

A northeast Washington collection of western larch seeds was stratified for 0, 10, 20, 40, and 80 days and incubated at 55, 64, and 73 °F. The germination percentage of filled seeds and speed and uniformity of germination were improved by long stratification, particularly at the lowest incubation temperature. Stratified seeds were also nursery sown in early April and May. Emergence occurred when mean air temperatures were low (48-54 °F), which indicated that long stratification should be beneficial in the nursery. First-year seedling height was closely related to date of emergence.

Keywords: Emergence, germination percent, germination rate, incubation temperature, stratification, seedling height.

Introduction

Seeds of larch species, with the exception of *Larix lyallii* Parl., are reported to have mild dormancy, which can vary within a species (Rudolf 1974). In genetic tests, prompt and complete emergence is desirable to reduce subsequent variability in plant size and to minimize selective effects in the nursery (Campbell and Sorensen 1984). The germination tests reported here were conducted preparatory to establishment of a nursery study on genetic variation in western larch. Successful germination and emergence of western larch (*L. occidentalis* Nutt.) requires seed pretreatment, or emergence will be slow and prolonged (Shearer and Tackle 1960). Several hydrogen peroxide and stratification treatments have markedly increased the rate of germination (Schmidt 1961, 1962; Shearer and Tackle 1960). The main purpose of my test was to obtain additional information about the effect of stratification pretreatment and incubation temperature on germination of western larch seed and to relate the results to temperature conditions in the nursery bed. Three experiments were conducted.

I. Seeds were stratified for five periods of different lengths. Mean germination rate, standard deviation (spread) of rate, and the percentage of germination were determined at three incubation temperatures.

II. Seeds were sown dry and unstratified once in autumn and sown stratified on two dates in spring. Mean dates of emergence were determined for the three sowing dates. Air temperatures were recorded between sowing and emergence in the spring. Seedling heights were measured at the end of the first year.

III. Seeds of western larch, ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), lodgepole pine (*Pinus contorta* var. *latifolia* Engelm.), and two sources of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were sown in autumn, and mean dates of emergence were compared.

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Materials and Methods

Seeds from a bulk lot of larch cones collected in northeast Washington in autumn 1987 were used in the test. Cones were processed and seeds extracted under standard conditions. Filled seeds were separated on an air column until x-ray examination indicated empty seed proportion <5 percent.

Experiment I

Seeds were counted into 60-seed lots, which were placed in small packets made from commercial bridal veil cloth. Packets were soaked for 24 hours in aerated water, then placed, together with moistened paper towels, in polyethylene bags that were sealed and placed in a walk-in cooler kept at 35-38 °F. Five stratification periods—0, 10, 20, 40, and 80 days—were used. Zero-day chilling was included because it has been reported that "seed of most larch species germinates fairly well without pretreatment" (Rudolf 1974). The time scale used for the remaining stratifications was based on the relation between logarithm of chilling period and germination rate being approximately linear for seeds of many tree species (Sorensen 1983).

Incubation temperatures were 55, 64, and 73 °F. Temperatures were constant day and night within about 2 °F. Because of the strong effect incubation temperature has on dormancy (Vegis 1963), temperatures were chosen to approximately span the range of spring nursery and greenhouse (for container culture) temperatures. Photoperiod was 12 hours; illumination was from a combination of incandescent and cool white fluorescent lamps.

Seeds were placed on filter paper in covered Petri dishes. The filter paper was placed on a thin layer of vermiculite saturated with a measured volume of distilled water. Sixty seeds were placed in each dish. All dishes went into the incubation chambers at about the same time. Five dishes of each stratification period were put in each of the three incubation chambers with each chamber set at a different temperature. Dishes were randomly assigned to positions on a shelf within each chamber.

The statistical model was (Milliken and Johnson 1984, p. 418),

$$X_{ijk} = M + A_i + C_j + B_j + (AB)_{ij} + e_{ijk},$$

where
X_{ijk} = observation for one dish;
M = grand mean;
A_i = fixed temperature effect;
C_j = random chamber effect, which was assumed = 0;
B_j = fixed stratification effect;
$(AB)_{ij}$ = fixed interaction effect;
e_{ijk} = random error (or dish effect);
i = 1, 2, 3 temperatures;
j = 1, 2, 3, 4, 5 stratification periods; and
k = 1, 2, 3, 4, 5 replications.

Table 1—Form of the analysis of variance of germination traits in experiment I[a]

Sources of variation	D.f.[b]	Expected mean squares
Total	74	
Stratification period:	4	$\sigma^2 + 15K_S^2$
0 vs. stratification	1	
Among stratifications	3	
Temperature	2	$\sigma^2 + 25K_T^2$
T x S:	8	$\sigma^2 + 5K_{ST}^2$
T x SO vs. 1-8[c]	2	
T x Among S1-8—	6	
Experimental error	60	σ^2

[a] Treatments included five stratification periods (0, 10, 20, 40, and 80 days) and three incubation temperatures (55, 64, and 73 °F). All effects were fixed.
[b] Degrees of freedom.
[c] 0 and 1-8 refer to the stratification periods, 0 and 10-80 days, respectively. Stratification periods of 10-80 days are treated as linear on the log scale.

Except for zero stratification, treatments were orthogonal. The planned analysis (table 1) was modified somewhat in application: so few seeds germinated at 55 °F after 0 and 10 days stratification and at 64 °F after 0 stratification that not all treatment levels were included in some of the analyses (see below). Seeds were classed as germinated when emerging root tips showed visible geotropism, usually when about 0.1 inch long.

Three germination traits were analyzed. (1) **Rate of germination** was determined for each Petri dish according to the procedure of Campbell and Sorensen (1979). Rate expresses daily development toward germination after entering incubation conditions; if 8 days are needed for a seed to germinate, the embryo developmental rate is 1/8 = 0.125 units per day. Higher rates mean fewer days to germinate. (2) Spread of germination in **standard deviations of rate** was determined from the calculation of rate (Campbell and Sorensen 1979, table 2). A large standard deviation means a wide spread in rates. (3) The test ended after 40 days in the incubation chambers. Ungerminated seeds were x rayed and **percentage of filled but ungerminated seeds** determined for each Petri dish. The percentages of ungerminated seeds are based on filled, healthy-appearing seeds. The values differed widely among treatments and were transformed to arc sine $\sqrt{\text{percentages}}$ before analysis of variance.

Because few seeds germinated at three combinations of stratification periods and incubation temperatures, germination rate and its standard deviation were analyzed in two groupings of stratification period (S) and incubation temperature (T): S = 20, 40, 80 days and T = 55, 64, 73 °F; and S = 10, 20, 40, 80 days and T = 64, 73 °F.

Experiment II

Dry, unstratified seeds were sown on November 9, 1987, and seeds stratified for 50 days were sown on April 1 and May 6, 1988, onto raised nursery beds at Corvallis, Oregon. Three seeds were sown per spot on a 3- by 4-inch spacing and covered with a shallow layer of granite grit and with bird screening. Treatments (dates of sowing), in row plots 4 seed spots long, were randomly placed in 12 blocks. Emergence was tallied every other day beginning as soon as the first emergent seedling was observed. Mean day of emergence and its standard deviation were calculated for the seeds in each treatment. Emergence data were not analyzed statistically.

Mean daily temperatures and degree hours above 40 °F from dates of sowing to mean dates of emergence were determined for the two spring sowings. A seedling was considered emerged when the seedcoat was above the ground surface or, if the seedcoat was temporarily held below ground, when the hypocotyl arched above the ground surface. For the autumn-sown seeds, a mean temperature for the 20 days preceding the mean date of emergence was calculated. Temperatures were obtained from a recording thermometer with a covered probe that was situated about 65 inches above ground level and 45 inches above the soil level of the raised beds. The probe and recorder were in the same section of the nursery, but about 75 feet from the test area. (A soil-temperature probe was installed in the test area, but malfunctioned. No information on soil temperature is available.)

Seed spots were systematically thinned (easternmost and westernmost seedlings removed) to one seedling per spot in the summer. Total height was measured after bud set in autumn 1988. Plot mean heights were analyzed by using the statistical model (Snedecor and Cochran 1967, p. 303),

$$X_{ij} = M + A_i + B_j + e_{ij} ,$$

where X_{ij} = mean height for one plot;
M = grand mean;
A_i = fixed date of sowing effect;
B_j = block effect;
e_{ij} = experimental error;
i = 1,2,3 dates of sowing; and
j = 1,...,12 blocks.

Inadvertently one plot was not sown. A missing-plot value was estimated (Snedecor and Cochran 1967, p. 317); degrees of freedom for experimental error and total were reduced by one.

Experiment III

This small test was established to compare emergence of western larch with several other western conifers. Other species and seed sources were:

1. Ponderosa pine, a mix of about 140 families from the east slopes of the Cascade Range and central Oregon.

2. Lodgepole pine, a comparable mix from the same region.

3. Douglas-fir, a mix of about 15 families from the west slopes of the Cascade Range, low to middle elevations, latitude 44°30'N. ± 30'.

4. Douglas-fir, a seed-orchard mix, of unknown family makeup, but from about the same area as in 3.

All seed lots were cleaned to <5 percent apparent defective seeds before sowing. Dry, unstratified seeds were sown on November 9, 1987, into 25 plots, 100 seeds per plot. Seeds were covered with granite grit, depth depending on seed size: 0.1 inch for larch and lodgepole pine, 0.2 inch for Douglas-fir, and 0.3 inch for ponderosa pine. The test area was also covered with two layers of Saran shade cloth, which allows water to pass but prevents rain splash.[1] (According to manufacturer's specifications, two layers of cloth pass 20 percent of full light.) Emergence was tallied every other day. Percentage of emergence, mean date of emergence, and standard deviation of date were determined for each plot.

Seed plots were randomly placed in a 5 by 5 arrangement, 5 plots per species or source. The statistical model was,

$$X_{ij} = M + A_i + e_{ij} ,$$

where X_{ij} = value for one plot;
M = grand mean;
A_i = fixed species (seed source) effect;
e_{ij} = error or random plot effect;
i = 1,2,3,4,5 species (seed sources); and
j = 1,2,3,4,5 repeats.

Results and Discussion

Stratification-period and incubation-temperature main effects were, in all cases, highly significant factors in determining amount, rate, and uniformity of germination (table 2).

Experiment I

Percentage ungerminated—Stratification requirements were strongly related to the temperature the seeds were incubated at. At 73 °F, almost 90 percent of the seeds germinated without stratification, and 100 percent of the seeds germinated with 10 or more days of stratification. At 55 °F, 40 days of stratification were necessary to obtain satisfactory germination (fig. 1).

Rate of germination—Mean rates are shown graphically in figure 2. Because other results show that germination rates tend to be normally distributed (Campbell and Sorensen 1979), mean rates are close to median rates. The reciprocal of the sample **mean rate**, therefore, can be regarded as an estimator of the **median time** to germinate. As shown on the time scale (y-axis, fig. 2), reducing temperature from 64 °F to 55 °F considerably prolonged the time to germination, particularly for stratification periods shorter than 40 days.

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[1] Use of a trade name does not imply endorsement or approval of any product by the USDA Forest Service to the exclusion of others that may be suitable.

Table 2—Components of variance for experiment I expressed as percentage of total variance, proportion of total sums of squares explained by single degree of freedom contrasts (R^2), and coefficients of variation for experimental error for germination traits of western larch seed

	Germination traits				
	Percent ungerminated;	Germination rate		Standard deviation of rate	
Treatments	S=0–80, T=55–73[a]	S=10–80, T=64–73	S=20–80, T=55–73	S=10–80, T=64–73	S=20–80, T=55–73
Components of variance:					
Stratification period	41***[b]	83***	43***	42***	15***
Incubation temperature	27***	7***	51***	49***	78***
Interaction	31***	8*	1	−2	−1
Error	1	7	5	11	8
R^2	0.986	0.921	0.936	0.859	0.903
Coefficient of variation (percent)	6.8	7.0	7.5	9.6	10.6

[a] S is the range of stratifications periods (0, 10, 20, 40, 80 days); T is the range of incubation temperatures (55, 64, 73 °F) included in the analysis.
[b] Significance levels are *p<0.05; ***p.

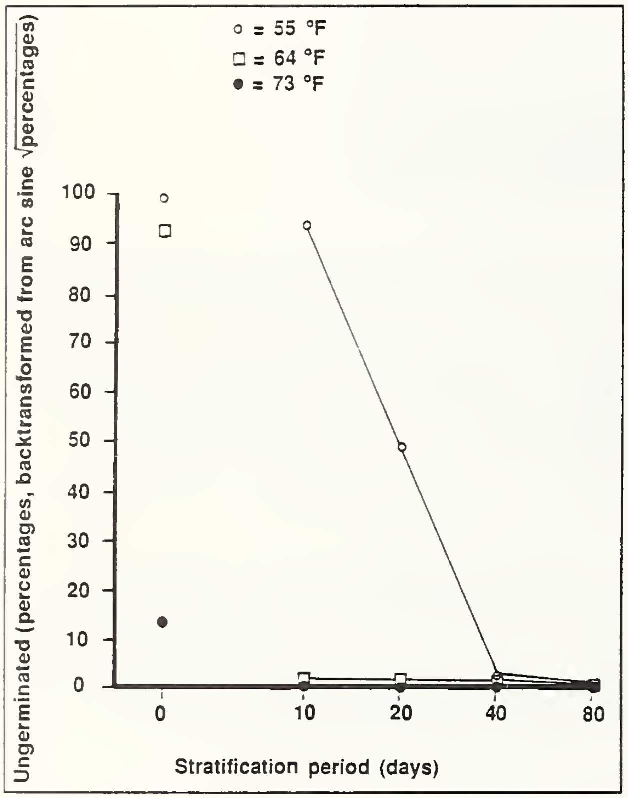

Figure 1—Effect of stratification period and incubation temperature in experiment I on percentage of filled western larch seeds not germinating within 40 days. Temperature was constant; photoperiod was 12 hours.

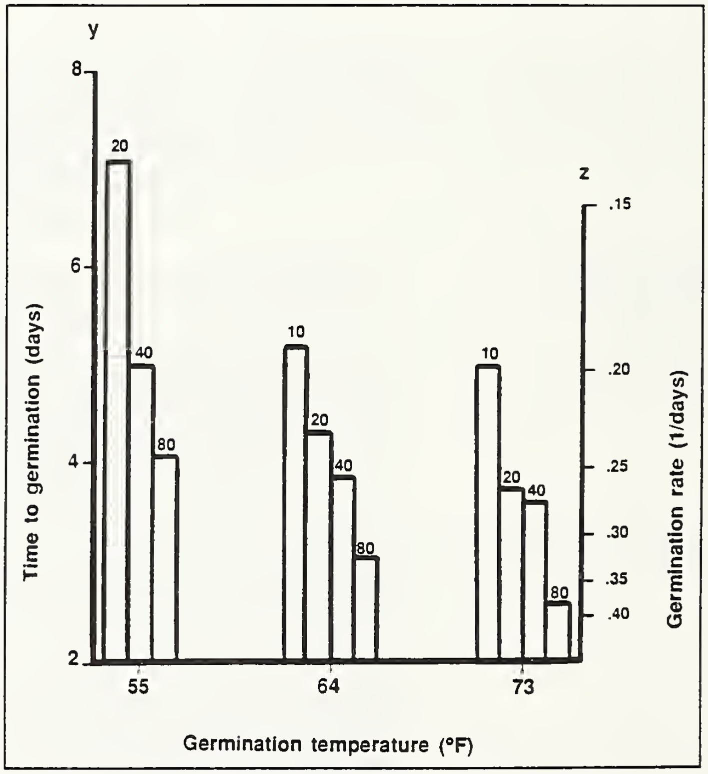

Figure 2—Effect of stratification period and incubation temperature in experiment I on median time to germination (y-axis) and mean germination rate (z-axis). There is no column for 10 days at 55 °F because of insufficient germination.

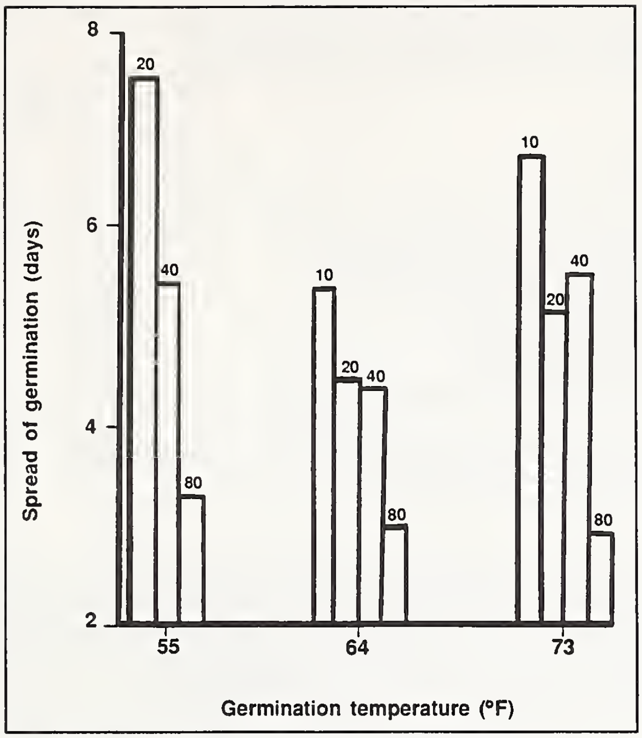

Figure 3—Effect of stratification period and incubation temperature in experiment I on spread of germination time. There is no column for 10 days at 55 °F because of insufficient germination. Numbers on the y-axis are the days within which the central 80 percent of the seeds germinated.

Spread of germination rates—Spread is presented in days, [D(54) – D(7)], where $D(1)<D(2)<\ldots<D(60)$, and D is the time to germinate for each of the 60 seeds in a dish. If less than 60 seeds germinated, equivalent numbers were used. This value approximates the time span within which the central 80 percent of the seeds germinate.

Spread of germination differed widely with stratification period and incubation temperature combinations (fig. 3). At temperatures as low as 55 °F, the uniformity of germination of seeds within a lot was strongly affected by stratification period (note that there is no column in fig. 3 for 10 for 55 °F because few seeds germinated with 10 days chilling). When seeds were stratified 80 days, germination was prompt and uniform at all temperatures used in this test.

In general, 40 days of chilling gave rapid germination; 80 days improved uniformity. Prompt and uniform germination and early seedling establishment are primary factors in consistently producing high-quality seedlings (Barnett 1985). Nevertheless, stratification as long as 80 days needs to be done with care. Metabolic activity in stratification generates some heat. To prevent germination of any seeds during long chilling, the temperature must be well controlled and packages or layers of seeds must be small enough that heat does not build up inside the package. Sometimes in nursery practice, planned sowings are delayed by bad weather. If this extends an already

Table 3—Effect of date of seed sowing on emergence and 1st-year height of western larch in experiment II

Traits	Date of sowing		
	Nov. 9	Apr. 1	May 6
Mean date of emergence	Mar. 4	Apr. 18	May 18
Spread of emergence (days)[a]	11	9	3
Emergence percent	71	88	89
Mean air temperature (°F)	41.9[b]	49.1[c]	54.0[c]
Degree-hours (°F)	—	43.20[c]	43.40[c]
First-year seedling height (inches)	10.0	6.8	4.6

[a] Days over which central 80 percent of germinant seedlings emerged.
[b] For 20 days preceding March 4.
[c] For period between sowing and mean date of emergence.

long chilling, germination of some seeds may occur in storage.[2] We have not stratified larch seeds beyond 80 days, but have experienced no germination in 35-38 °F stratification up to 80 days. If delayed sowing is a potential problem, chilling for 75-70 days could be used. With long delays seed might be surface dried and held at 35-38 °F (Belcher 1982; see footnote 2).

The germination of low-vigor seeds (seeds extracted from cones that were collected when still immature or that were handled roughly during cleaning) may be reduced a few percentage points by long chilling (Allen 1960, Sorensen 1980), but the advantage of rapid, uniform germination is retained.

Experiment II

Results of the autumn vs. spring sowings showed lower emergence percentages for the autumn sowing. This was possibly due to rain splash and consequent seed loss. In another test (see below) where seeds were protected, emergence after autumn sowing was similar to that of spring sowings in experiment II.

Autumn-sown seeds emerged when air temperatures were still low (table 3). Mean days to emergence were 18 and 12 for seeds sown April 1 and May 6, respectively. Accumulated Fahrenheit degree-hours above 40 °F were slightly above 4300 for both sowing dates (table 3).

Degree-hours to emergence in the nursery bed were compared with those for germination in the incubator. The 55 °F incubation temperature-40 day stratification was the most comparable. At that combination, the mean time to germination was 5.0 days (fig. 2). Accumulated heat units in the incubator were 1730 °F hours, or about 40 percent of that required for emergence in the nursery at a slightly lower mean temperature.

[2] Personal communication, Y. Tanaka. Forest Nursery Ecologist, Western Forestry Research Center, Weyerhaeuser Company, Centralia, WA 98531.

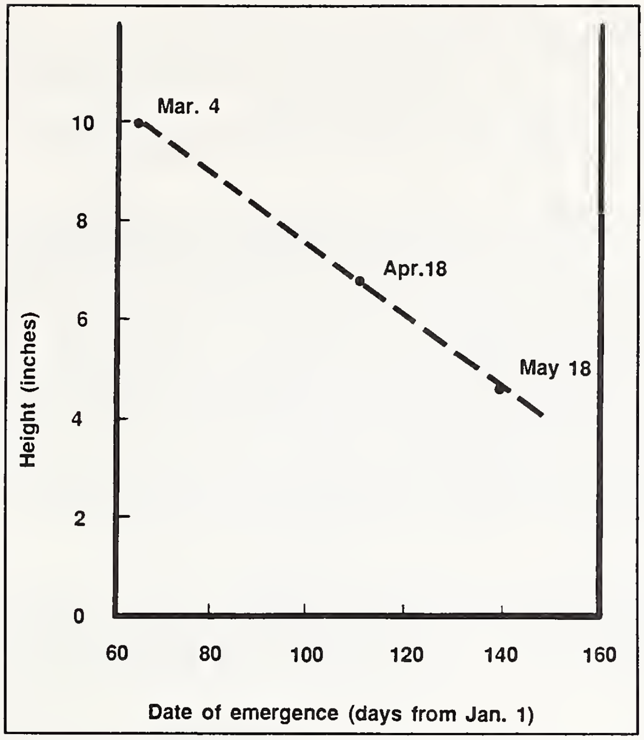

Figure 4—Relation between mean date of emergence and mean first-year seedling height in western larch in experiment III. Sowing dates were November 9 (mean date of emergence, March 4), April 1 (April 18), and May 6 (May 18).

Because western larch seedlings elongated late into the summer irrespective of emergence date, sowing date had a highly significant effect on first-year height ($F = 10.79$; d.f. = 2,21; $p = 0.0006$). Regression of mean height on date of emergence (fig. 4) indicated that for each day emergence was delayed between March 4 and May 18, mean first-year height was reduced by 0.07 inch.

Experiment III

Of the four species sown in autumn, western larch emerged earliest, March 7 vs. March 12, 14, and 22 for ponderosa pine, lodgepole pine, and Douglas-fir, respectively. The rapid germination or emergence observed in the other experiments was reasonable for western larch seed originating in northeastern Washington. Emergence averaged 87 percent and did not differ among species. Mean air temperature for the 20 days prior to March 7 was 41.7 ^{o}F. This was cold, but did include several clear days with temperatures >60 ^{o}F in late February and again in the second week of March. A frost of 22 ^{o}F was recorded the night of March 14/15. Early germinant seedlings, which had only cotyledons exposed on this date, were not damaged. No frosts below 27 ^{o}F were recorded after March 19, and no frost damage was observed.

Conclusions

If the seed sample used in these tests was representative of western larch in the center of its range, then:

1. Larch germinants emerge early and relatively uniformly when sown in autumn.

2. First-year growth in the nursery is responsive to the time of emergence.

3. Stratification should be only marginally necessary for germination under standard test conditions of a 86 °F day/68 °F night thermoperiod.

4. Short stratification should be sufficient for germination in a heated or warm glasshouse, but long stratification will improve uniformity.

5. For sowing at spring nursery temperatures, stratification is necessary. To achieve rapid and uniform germination, long stratification (60 days or more) is recommended with good control of the stratification temperature.

Acknowledgments

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Literature Cited

Allen, G.S. 1960. Factors affecting the viability and germination behavior of coniferous seed. IV: Stratification period and incubation temperature, *Pseudotsuga menziesii* (Mirb.) Franco. Forestry Chronicle. 36: 18-29.

Barnett, J.P. 1985. Techniques for improving the performance of southern pine seeds in nurseries. In: South, David B., ed: Proceedings of the international symposium of nursery management practices for the southern pines; 4-9 August 1985; Montgomery, AL. Auburn University, AL: Auburn University, Agricultural Experiment Station: 102-112.

Belcher, E.W. 1982. Storing stratified seeds for extended periods. Tree Planters' Notes. 33(4): 23-25.

Campbell, R.K.; Sorensen, F.C. 1979. A new basis for characterizing germination. Journal of Seed Technology. 4: 24-34.

Campbell, R.K.; Sorensen, F.C. 1984. Genetic implications of nursery practices. In: Duryea, Mary L.; Landis, Thomas D., eds. Forest nursery manual production of bareroot seedlings. Boston: Martinus Nijhoff/Dr. W. Junk Publishers: 183-191.

Milliken, G.A.; Johnson, D.E. 1984. Analysis of messy data. Volume 1: Designed experiments. Belmont, CA: Lifetime Learning Publications. 468 p.

Rudolf, P.O. 1974. *Larix* Mill. In: Schopmeyer, C.S., comp. Seeds of woody plants in the United States. Agric. Handb. 450. Washington, DC: U.S. Department of Agriculture, Forest Service: 478-485.

Schmidt, Wyman C. 1961. Effects of some treatments on the dormancy of western larch seed. Missoula, MT: Montana State University. M.S. thesis.

Schmidt, Wyman C. 1962. Rapid viability tests for western larch seeds. Proceedings of the Montana Academy of Sciences. 21: 26-32.

Shearer, Raymond C.; Tackle, David. 1960. Effects of hydrogen peroxide on germination in three western conifers. Res. Note 80. Ogden UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 4 p.

Snedecor, G.W.; Cochran, W.G. 1967. Statistical methods. 6th ed. Ames, IA: The Iowa State University Press. 593 p.

Sorensen, Frank C. 1980. Effect of date of cone collection and stratification period on germination and growth of Douglas-fir seeds and seedlings. Res. Note PNW-346. Portland OR: U.S. Department of Agriculture, Forest Service, Pacific Northwest Forest and Range Experiment Station. 11 p.

Sorensen, Frank C. 1983. Relationship between logarithms of chilling period and germination or bud blush rate is linear for many tree species. Forest Science. 9: 237-240.

Vegis, A. 1963. Climatic control of germination, bud break, and dormancy. In: Evans, L.T., ed. Environmental control of plant growth. New York: Academic Press: 265-287.

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